Chapter 13
The Analysis of the Functions of Human B and T Cells in Humanized NOG Mice

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Over the past two decades, reconstitution of human hematopoietic and immune systems in mice has been explored using several severely-immunodeficient mouse models including NOD/scid, NOG, NSG, or BRG [1, 2]. These parental mouse strains have been modified extensively by introducing human genes or replacing mouse genes with the corresponding human genes [3]. These models are currently utilized in many fields of research, and the establishment of such humanized mice has resulted in various advances including engraftment of human hematopoietic stem cells (HSCs) [4], differentiation of multiple lineages of human cells [5, 6], and enhancement of immune responses [7–9]. However, for improved application of this technology, it is important to recognize several immunological features intrinsic to humanized mice and to consider carefully the choice of mouse strains. This chapter describes the properties of human B and T lymphocytes that develop and constitute major subpopulations in NOG mice transplanted with HSC (huHSC-NOG).

B lymphocytes Development of human B lymphocytes in NOG mice can be detected in peripheral blood at ~4–6 weeks after HSC transplantation [10]. In conventional NOG mice, most human $CD45^+$ cells in the peripheral blood are $CD19^+$ B cells, and this population rapidly increases 2–3 months after HSC transplantation. However, as development of human T cells becomes evident after 3 months, the frequency of B cells decreases gradually compared to those observed at early time points.

The phenotype of human B cells in huHSC-NOG mice generally resembles that of B cells from healthy human donors [11]. In spleen, $CD19^+$ cells comprise mainly IgM^+IgD^- and IgM^+IgD^+ cells. The phenotype of the IgM^+IgD^- population is similar to that of immature B cells in bone marrow, whereas the IgM^+IgD^+ B cells seem to represent more mature B cells. Along with T-cell differentiation, the phenotype of

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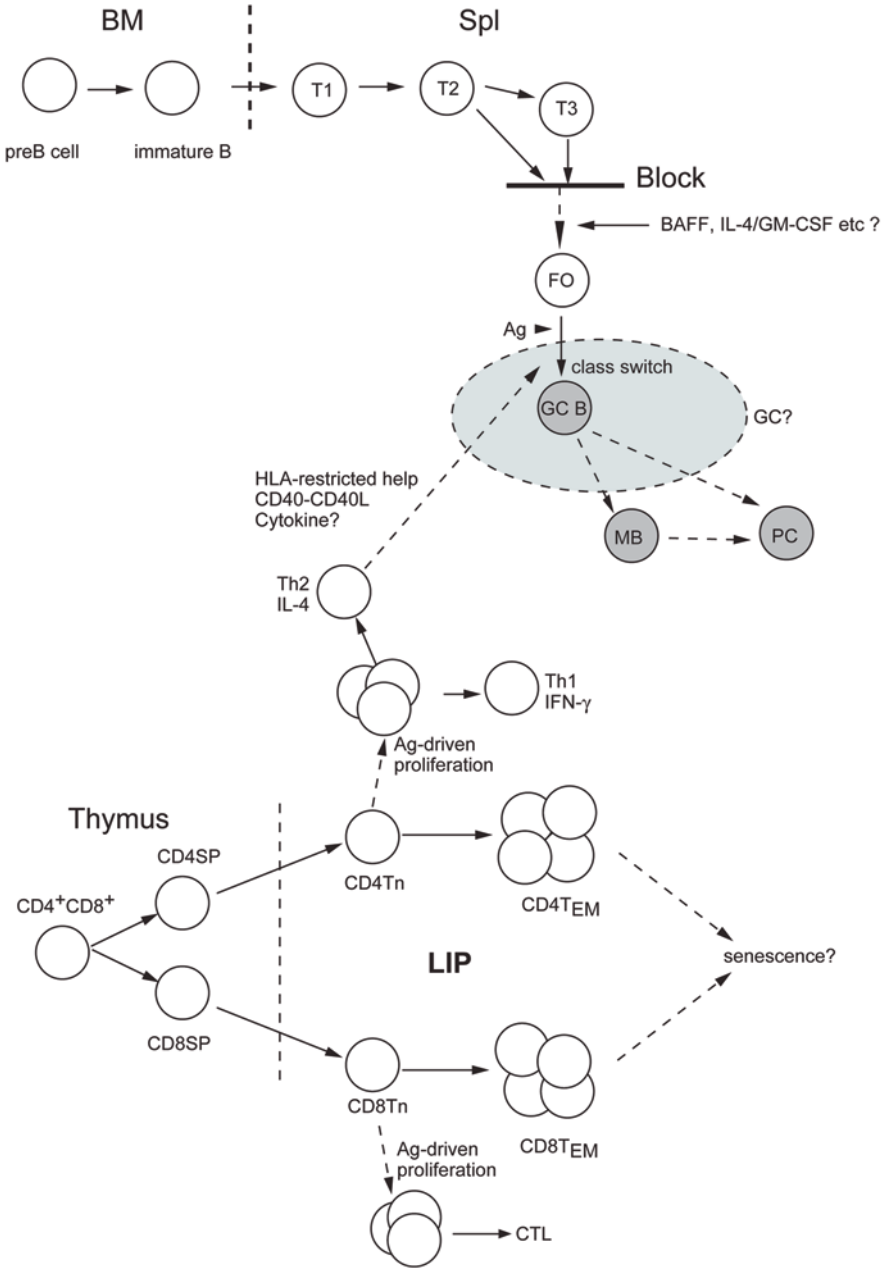

Fig. 13.1 Schematic of immune responses by human B and T cells in humanized mice**.** Human B cells develop in mouse bone marrow (*BM*). However, differentiation of most B cells is blocked around the transitional stages (T1–T3) before reaching mature follicular cells (FO). T cells are positively selected in the thymus in a process that depends largely on the mouse major histocompatibility complex (MHC). After migrating into the periphery, T cells are subjected to lymphopenia-induced proliferation (*LIP*), which might result in loss of T-cell function. In the case where human leukocyte antigens (HLAs) are

IgM^+IgD^+ B cells becomes more mature, resembling that of follicular B cells from normal humans [12], and a few $CD27^+$ memory B cells appear in the late period after HSC transplantation. However, there are significant differences between human B cells from donors and human B cells expressed in huHSC-NOG mice. The most prominent differences are the high CD5 and low CD21 expression levels in B cells from huHSC-NOG mouse, suggesting incomplete differentiation of human B cells in humanized mice (Fig. 13.1) [13, 14].

Regarding immunological function, human B cells from huHSC-NOG mice produce IgG in vitro in response to mitogen and anti-CD40 antibody stimulation in the presence of IL-21, suggesting that the class-switching molecular machinery is functional [14]. In addition, in vitro stimulation of human B cells from huHSC-NOG mice induces the expression of activation-induced deaminase (AID), a critical molecule for class-switching and somatic hyper mutation (SHM) [14].

B-cell function in hu-HSC NOG mice has been evaluated in a number of studies in vivo, with differing results. Initial experiments demonstrated that B cells could respond to various antigens, and that the specific antibodies produced were IgM-dominant, with rare instances of IgG [15, 16]. Thus, it was assumed that human T cells and B cells did not interact; i.e., most human T cells were positively selected by mouse major histocompatibility complex (MHC) in the mouse thymus and could not recognize peptide-human leukocyte antigen (pHLA) complexes on human B cells. To circumvent the mismatch of MHC-restricted T cells and pHLA on B cells, several HLA-DR transgenic NOG or NSG mice were generated [8, 9]. Reconstitution of these mice with HSC revealed that an IgG response was possible when the HLA-DR haplotype was matched between donor HSC and recipient mice, suggesting that humoral immune responses can be mediated in human-HSC transferred mice, albeit with limited magnitude.

Affinity maturation of antibodies is another important aspect of humoral immunity. However, due to the weak IgG response in huHSC-NOG mice, affinity maturation of antibodies has not been detected in humanized mice. It should be noted that conventional humanized mice have poorly organized follicular structure in the secondary lymphoid organs and germinal centers (GC; Fig. 13.1). Given the key role of GCs in B-cell response, this defect raises the concern of whether naive B cells can differentiate into memory B cells or plasma cells in humanized mice in a manner similar to differentiation in the human lymph node (LN) or spleen. These potential similarities require further clarification.

T lymphocytes Human T cells appear in the peripheral blood in huHSC-NOG mice at about 3 months post-HSC transplantation [10]. However, human T cells do not develop in athymic nude NOG mice (nu/nu NOG) (T.T. unpublished data), suggesting that the thymus in the recipient mouse is required for T-cell development. Most human thymocytes in huHSC-NOG mice are positively selected by mouse MHC,

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matched between the hematopoietic stem cell (HSC) and recipient mouse, T cells can be activated in an HLA-restricted manner and differentiate into effector cells, which can support class-switching in B cells. No clear evidence has suggested germinal center (*GC*) formation or differentiation of antigen-specific memory B (*MB*) cells and plasma cells (*PC*), which are represented by *shading*

as evidenced by the fact that the number of $CD8^+$ or $CD4^+$ human T cells is reduced in beta-2-microglobulin (β2m)- or I-Aβ-deficient NOG mice [14], respectively. The development of a few human T cells in these MHC-deficient mice suggests that human thymocytes could differentiate into mature T cells in a thymic epithelial cell (TEC)-independent manner. Developing thymocytes in close proximity might provide HLA signals to facilitate maturation [17].

T-cell-mediated immunity in huHSC-NOG mice has been investigated in several reports using virus infection, including immune responses against Epstein-Barr virus (EBV) [15, 18]. Interestingly, mice that harbored solely human B cells developed B-cell lymphoma, whereas animals with both human B and T cells were protected from the development of B-cell lymphoma [15, 19]. In these animals, EBV-specific $CD8^+$ cytotoxic T lymphocytes (CTL) were detected by the specific HLA tetramer [20], and they produced cytokines in response to in vitro exposure to an EBV-transformed autologous B-cell line [18, 19]. These results suggest that human $CD8^+$ T cells developed in humanized mice have sufficient function to eradicate EBV. Considering that positive selection of human thymocytes depends on mouse MHC, it is curious that human $CD8^+$ T cells in huHSC-NOG mice can recognize EBV-derived antigens presented on HLA. One explanation is that a significant number of human $CD8^+$ T cells are positively selected through a TEC-independent mechanism, as described above. Recently, class I-HLA (HLA I)-expressing NSG mice were developed. In these animals transplanted with HLA haplotype-matched HSC, human T cells showed much clearer HLA-restricted immune responses, suggesting functional human $CD8^+$ T cells are maintained in the mouse environment [7, 19].

Regarding $CD4^+$ T cells, initial experiments using conventional NOG mice failed to show evidence of humoral immune responses since antigen-specific IgG is rarely produced by immunization or infections. HLA-DR-expressing transgenic NSG or NOG mice were capable of mounting successful IgG responses with HLA-matched HSC-transplantation, suggesting that human $CD4^+$ T cells in humanized mice can be activated in an antigen-specific manner and are able to exert helper function to B cells through pHLA and TCR interactions [8, 9]. In our hands, T cells from HLA-DR4 transgenic I-$A\beta^{-/-}$ NOG mice differentiated into IFN-γ-producing Th1 cells or IL-4-producing Th2 cells in response to in vitro stimulation (T.T., manuscript in preparation). Thus, human $CD4^+$ T cells in the mouse environment maintain the ability to differentiate into various lineages of effector cells. Likewise, differentiation into other effector lineages such as Th-17 or inducible regulatory T cells (iTreg) would be possible when the appropriate cytokine milieu is provided in mice.

It should be emphasized that T-cell homeostasis in humanized mice is not physiologically similar to that in normal humans. For example, a study using BRG mice showed that the human T cells were quickly labeled by BrdU [21]. In addition, the $CD45RA^+CD62L^{hi}$naive (Tn) T-cell phenotype in huHSC-NOG mice rapidly changed to the $CD45RO^+CD62L^{lo}$ effector/memory (T_{EM}) phenotype to produce abundant IFN-γ[9], and the accumulated T_{EM}-like cells did not proliferate or produce IL-2 in response to in vitro stimulation [14]. These studies suggest that human T cells in humanized mice are under strong pressure of lymphopenia (Fig. 13.1). Under extreme lymphopenic conditions, such as those found in NOG mice, a few human

T cells emigrate from the atrophic thymus and massively proliferate in response to an excessive amount of antigenic and cytokine signals. This lymphopenia-induced proliferation might be responsible for the reported impairment of T-cell function in conventional huHSC-NOG mice. Importantly, the phenotypes of human T cells in humanized mice are similar to those in patients with impaired thymopoiesis who had received cord-blood transplantation [22].

The bone marrow/liver/thymus (BLT) model might provide clues to the necessary components for reconstitution of a naive T-cell pool in huHSC-NOG mice [23]. In this animal, the T-cell phenotype resembles that of normal human T cells; i.e., a relatively high frequency of Tn cells is maintained. One prominent difference between the NSG- or NOG-based model and the BLT mouse is the size of the thymus. As mentioned previously, atrophy of the thymus in NOG mice is so severe that the number of human thymocytes in huHSC-NOG mice does not usually exceed 10^7. In contrast, the thymus/liver (Thy/Liv) organoid transplanted in a kidney capsule contains more than 10^8 cells [24]. Therefore, the enormous supply of T cells from the Thy/Liv organoid might help maintain Tn cells in BLT mice. Another difference between the two models is the presence or absence of LNs. LN development and LN number are significantly impaired in NSG or NOG mice [25], whereas NOD/scid mice, on which BLT mice are generated, have a normal number of LNs. This difference is attributable to the γc-deficiency in the former since the absence of IL-7-signal causes a significant decrease in lymph-tissue inducer cells (LTi) [26]. Considering that LNs produce growth factors including IL-7, the decreased LN number may affect human T-cell homeostasis. Thus, restoring LN development in γc-deficient mice is an intriguing approach to improving the naive T-cell pool.

The quality of quasi-human immune systems in humanized mice has been improved markedly by the introduction of human genes. Regarding acquired immunity, it is noteworthy that the development of human myeloid cells, including dendritic cells or macrophages, was greatly enhanced in human TPO-knock-in (KI) mice [6], human IL-3/GM-CSF KI mice [5] and IL-3/GM-CSF transgenic mice [27]. These myeloid cells will support human lymphocyte function. Additionally, various HLA-transgenic mice are useful for inducing HLA-restricted immune responses in the mouse environment. In the near future, integration of these multiple strains will enable the recapitulation of human immune responses in the mouse environment. Development of these animal models may lead to therapeutic approaches to the treatment of chronic diseases such as HIV-infection or autoimmune diseases.

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